Figure 5.3 (A) Genomic λclone E2 digested with SacI (lane 2) and Marker (lane 1); (B) clone pPIMP 770 digested with SacI liberating the 12 kb insert (lane 1).
Figure 5.4 (A) pPIMP 827 digested with SacI showing 4.8 kb insert (lane 2), and marker (lane 1); (B) pPIMP 833 (lane 1), pPIMP 834 (lane 2), pPIMP 936 (lane 3) and pPIMP 937 (lane 4) digested with SacI showing different sized inserts and marker (lane 5); (C) pPIMP 827 (lane 1) pPIMP 834 (lane 2), pPIMP 936 (lane 3), pPIMP 937 (lane 4) digested with SacI and marker (lane 5) separated in agarose gel for Southern blotting; (D) Southern blot probed with OsAMT1;1 showing respective hybridizing bands in pPIMP 827 (lane 1) and pPIMP 834 (lane 2).
Figure 5.5. Sequencing strategy for *OsAMT1;1* (λclone E2) from clone pPIMP770. Sequence PISE 52 with internal primer OsAMT1;1 -1698*, PISE 54 with internal primer OsAMT1;1 -2058*, PISE 60 with internal primer OsAMT1;1-864*, PISE 61 with internal primer OsAMT1;1-1143*, PISE 55 with internal primer OsAMT1;1-44* of *OsAMT1;1* cDNA, PISE 71 with internal primer OsAMT1;1-90 (*NcoI* overhang), PISE 80 with internal primer SE71-414* and PISE 82 with internal primer SE79 -597*.
Figure 5.6 PCR amplification of an *AMT1* gene product from clone E2. A PCR product of 2.04 kb was amplified from λ E2, using OsAMT1;1-44* forward primer and OsAMT1;1-2058* reverse primer.
Figure 5.7 Evaluation of 5' and 3' sequences of pPIMP577.

(A). *Oryza sativa* putative ammonium transporter *OsAMT1p* (*OsAMT1*) mRNA, complete cds, Length = 2046.
Score = 599 bits (302), Expect = e-169 Identities = 311/314 (99%) Strand = Plus / Plus

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113
(B). *Oryza sativa* putative ammonium transporter *OsAMT1p* (*OsAMT1*) mRNA, complete cds

Length = 2046

Score = 1090 bits (550), Expect = 0.0  Identities = 594/605 (98%), Gaps = 4/605 (0%)  Strand = Plus / Minus

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114
Figure 5.8 Sequencing strategy for OsAMT1;2 (λclone A3 & J) from the subclones pPIMP321 and pPIMP827. Sequence PISE 46 with universal reverse primer, PISE7 with M13 universal forward primer, PISE17 with internal primer SE7-696*, PISE 20 with internal primer SE17-658*, PISE23 with internal primer SE 20-649*, PISE 28 with internal primer SE 23-423+, PISE 29 with internal primer S 8-559+, PISE 8 with M13 universal reverse primer and PISE 45 with universal forward primer.
Figure 5.9 The sequences of isolated genomic clones of *OsAMT1* gene family, methionine codons are in bold and italicised.

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Consensus ~~~~~~~~~~~~~ ~~~~~~~~~~~~~ ~~~~~~~~~~~~~ ~~~~~~~~~~~~~ ~~~~~~~~~~~~~
Figure 5.10 Sequencing strategy for OsAMT1;3. (λ clone K) from the sub clone pPIMP834. Sequence PISE47 with M13 universal reverse primer, PISE 57 with clone K internal primer SE47-538⁺, PISE 62 with internal primer SE57-565⁺, PISE 69 with internal primer SE62-624⁺ and PISE 75 with internal primer S69-579⁺.
Figure 5.11 A. Phylogenetic tree of plant ammonium transporters. ORF were aligned using PILEUP. The gene bank accession numbers for the sequences used are as follows: *At AMT1;1* (X75879), *AtAMT1;2* (AF083036), *AtAMT1;3* (AF083035), *AtAMT2* (AF 182039), *AtAMT2G* (AC004683), *AtAMT3G* (AL035709), *AtAMT3* (ATF 16A16), *LeAMT1;1* (X92854), *LeAMT1;2* (X95098), *LeAMT1;3* (AF 118858) *OsAMT1;1* (This thesis), *OsAMT1;2* (This thesis), *OsAMT1;3* (This thesis). B. Comparison of the known plant AMT ORF sequences. ORF sequence similarities between all pairs of available Ammonium transporter sequences.
Figure 5.12 Construction of promoter-gus fusions. (A) A 3.6 kb PCR product amplified with the forward primer SE74-668+ and the reverse primer OsAMT1;1-90′; (B) PCR product was digested with HindIII and Ncol to produce three fragments including a 2.5 kb HindIII/Ncol fragment; (C) construct pPIMP920 showing the 2.5 kb promoter region from OsAMT1;1 gene linked to uidA; (D) construct pPIMP841 showing 2.38 kb promoter region from OsAMT1;2 gene linked uidA; (E) construct pPIMP934 showing the 1.4 kb promoter region from OsAMT1;3 gene linked to uidA.
Figure 5.13. The nucleotide sequences of the rice ammonium transporter gene OsAMT1;1 (A), OsAMT1;2 (B) OsAMT1;3 (C). The deduced amino acid sequence is shown underneath in single letter code. The symbol * indicates the termination codons. Proposed ATG initiation codons are bold and italics. Putative GATA sequences are underlined, TATA and CAAT sequences are in bold and underlined, and possible transcription termination sequences (arrow and underlined) are indicated. The GenBank accession number for these sequences are Bankit No. 348508, 348512 and 348516 for OsAMT1;1, OsAMT1;2 and OsAMT1;3 respectively.

(A). OsAMT1;1

...
Table 5.1 Percent identity of nucleotide and peptide among three members of OsAMT1 family and peptide percent identity between OsAMT1;1 cDNA and three genomic members of OsAMT1.

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CHAPTER 6

GENERAL DISCUSSIONS
6.1 Diversity and Evolution of the AMT

The uptake and assimilation of inorganic nitrogen onto carbon skeletons have marked effects on plant productivity, biomass and crop yield (Lawlor et al., 1989). There are various sources of NH$_4^+$ for plants, including absorption from soil, NO$_3^-$ reduction, and as a by product of photorespiration, protein degradation or amino acid catabolism (Mack, 1995). The existence of multiple ammonium transporters in plants presumably allows efficient use of different NH$_4^+$ sources. Recent studies have thrown some light on the molecular identities of different ammonium transporters. There are at least two gene families encoding for NH$_4^+$ uptake proteins in Arabidopsis, including AMT1 and AMT2 (Sohlenkamp et al., 2000). Three AMT1 family members have been isolated in Arabidopsis and in tomato (Gazzarrini et al., 1999; Lauter et al., 1996; Ninnemann et al., 1994; Von Wiren et al., 2000). Yeast has three NH$_4^+$ transporters, encoded by the Mep1, Mep2 and Mep3 genes of the same family (Marini et al., 1997; Marini et al., 1994). During the course of this study genomic clones of three putative rice ammonium transporters were isolated, including the one identical to the published OsAMT1;1 cDNA (Von Wiren et al., 1997). The completion of Arabidopsis genome sequencing has revealed at least five AtAMT1 family members and one AtAMT2. As rice genome sequencing progresses it is likely that other ammonium transporters will be identified. The first bacterial NH$_4^+$ transporter gene, Amt was isolated from Corynebacterium glutamicum (Siewe et al., 1996). The AMT protein of C. glutamicum shows significant identity to the MEP1 and MEP2 of S. cerevisiae (Marini et al., 1994) and AtAMT1;1 (Ninnemann et al., 1994). It has now been recognized that ammonium transporters are encoded by multigene families in plants, reflecting the fact that different transporters are utilized under different physiological conditions. However, it is still not clear about the functional redundancy among different transporters. If there is significant redundancy then mutation or knockout at the DNA level in a single transporter gene may have little or no obvious phenotype.

6.2 Function of AMT

Initial data regarding the possible physiological roles of specific plant ammonium transporters came from gene expression studies. Most of the plant ammonium transporter genes so far described including OsAMT1;1, are expressed both in roots and shoots. Exceptions are AtAMT1;2 and AtAMT1;3 which are expressed only in roots and LeAMT1;3 which is expressed only in shoots (Gazzarrini et al., 1999; Lauter et al., 1996; Ninnemann et al., 1994; Sohlenkamp et al., 2000; Von Wiren et al., 2000). In most cases transcript levels are higher in
roots than in shoots, suggesting a possible role of the cognate proteins in acquisition of NH$_4^+$ from growth medium (Ninnemann et al., 1994; Von Wirén et al., 2000). All three tomato NH$_4^+$ transporters and the Arabidopsis AtAMT1;1-3 complemented a yeast mutant defective in NH$_4^+$ uptake confirming their role in ammonium uptake (Gazzarrini et al., 1999; Lauter et al., 1996; Ninnemann et al., 1994; Von Wirén et al., 2000). It has been shown in bean and rice that NH$_4^+$ is partially assimilated at root hairs (Cullimore, 1991; Ishiyama et al., 1998). Two related NH$_4^+$ transporter genes LeAMT1;1 and LeAMT1;2 are preferentially expressed in root hairs. Promoter-reporter gene expression and protein immunolocalization studies showed high expression of GS1 and NADH-GOGAT in root hairs as well as in epidermal and exodermal root cells, respectively (Watson and Cullimore, 1996). Finally, transcriptional upregulation of AtAMT1;1 and LeAMT1;1 were closely correlated with enhanced $^{15}$NH$_4^+$ influx after plant transfer to nitrogen free nutrient solution (Gazzarrini et al., 1999; Von Wirén et al., 2000). It appears that plant N nutritional status and substrate availability differentially control transcription of some AMT genes. Thus transcript levels of AtAMT1;1 and LeAMT1;1 increased rapidly during nitrogen deprivation and decreased rapidly in response to high nitrogen supply (Gazzarrini et al., 1999; Von Wirén et al., 2000). On the other hand, transcript levels of AtAMT1;2 & AtAMT1;3 did not increase significantly following nitrogen starvation (Gazzarrini et al., 1999). Similarly, OsAMT1;1 showed constitutive expression under all nitrogen conditions (this thesis). Rather than decreasing or remaining the same, transcription of LeAMT1;2 increased after NH$_4^+$ or NO$_3^-$ supply (Von Wirén et al., 2000). It seems likely that AMT genes which are up-regulated in response to N limitation encoded transporters that enable NH$_4^+$ uptake at low concentration. This hypothesis is supported by the high affinity for ammonium that some of these proteins show when expressed in yeast (Gazzarrini et al., 1999). In contrast, NH$_4^+$-inducible LeAMT1;2 expression suggests a function for the cognate protein in NH$_4^+$ uptake at higher external concentrations, that could serve in retrieval of NH$_4^+$, thus compensating constant NH$_4^+$ efflux from roots derived from amino acid catabolism (Feng, Volk, and Jackson, 1998). Constitutive expression of OsAMT1;1 in roots as observed in this study, suggests that OsAMT1;1 may be involved in ammonium uptake under N limiting conditions and/or recovery of ammonium lost from roots during metabolism. Many species that normally use NO$_3^-$ also have an efficient system(s) for absorbing NH$_4^+$ which is constitutively expressed at high N levels (Forde and Clarkson, 1999). Where such species are presented with a mixed NO$_3^-/NH_4^+$ source, NH$_4^+$ is absorbed more rapidly, for example in perennial rye grass and barley (Clarkson et al., 1986; Macduff and Jackson, 1991). Such a role
requires a plasma membrane location for these transporters which has not yet been demonstrated.

The expression of AMTs in leaves suggests that they may be involved in photorespiratory ammonium transport (Gazzarrini et al., 1999; Sohlenkamp et al., 2000). Low atmospheric CO₂ concentrations stimulate photorespiration leading to release of NH₃ from glycine in mitochondria (Ogren, 1984). As the reassimilation of photorespiratory NH₃ is catalysed by glutamine synthetase localized in the chloroplast (Wallsgrove et al., 1987), it must be transported across the membranes of both organelles. However, nothing is known about ammonium transport in these organelles. One recent study supported the idea of involvement of members of AMTs in uptake and/or retrieval during photorespiration (Von Wieren et al., 2000). It has been shown that at elevated CO₂ levels, transcripts of LeAMT1;2 and LeAMT1;3 slightly decreased in leaves with simultaneous strong decrease in chloroplast glutamine synthetase (GS2) and photorespiratory serine hydroxymethyl transferase (Von Wieren et al., 2000). The authors proposed that elevated CO₂ levels decrease photorespiratory NH₃ evolution, which in turn, repressed LeGS2 transcription as well as LeAMT1;2 and LeAMT1;3. It was suggested that these transporters, particularly LeAMT1;2 may be involved in the retrieval and import of photorespiratory NH₃ escaping from mitochondria. This is because accumulation of LeAMT1;2 transcripts is higher during daylight when photorespiration peaks and when a considerable amount of NH₄⁺ is translocated in the xylem (Husted and Schjoerring, 1995). Although the diurnal variation in OsAMT1;1 expression or its expression patterns at different CO₂ levels is not known, its expression in leaves indicates that it may play a similar role as LeAMT1;2. Expression studies of AMTs under different conditions suggest that the transporters are involved in NH₄⁺ uptake from soil and ammonium recycling in shoots. To fulfill such roles, ammonium transporters must be located in the plasmamembrane and possibly other membranes, such as the inner membrane of the chloroplast. Unfortunately, no information is yet available on the location of AMT proteins in plant cells. Another serious gap in our understanding about the function of AMTs is that there are no reports of any AMT mutants or transgenic lines with altered AMT expression. The data presented in this study is the first report of alteration of expression of an AMT in plants.

6.3 Transgenic Approach to Study the Function of OsAMT1;1

Expression of the OsAMT1;1 gene in an antisense orientation (to inhibit gene expression) or in a sense orientation (to enhance gene expression) in transgenic plants was an obvious approach
to understand its physiological function. Due to the progress of foreign gene delivery and expression, transgenic rice is useful model to investigate gene functions (McElroy and Brettell, 1994; Upadhyaya et al., 2000). The sense and antisense OsAMT1;1 constructs (pPIMP161 and pPIMP145) produced in this work were introduced into the two rice cultivars Taipei 309 and Jarrah by using a well established Agrobacterium-mediated transformation system (Upadhyaya et al., 2000; Wang et al., 1997). Transformation efficiencies were higher in Taipei 309 compared to Jarrah. Differences in transformation efficiencies among rice cultivars has been reported previously (Upadhyaya et al., 2000). A small number of transgenic plants were regenerated from calli transformed with antisense OsAMT1;1 construct (pPIMP145) for cultivar Taipei 309. No antisense transgenic plants were recovered for cultivar Jarrah. The number of sterile plants was higher in antisense transgenic lines compared to sense transgenic lines. Therefore, it appears that there could be some lethal effect of the antisense transgene in rice. Overall, results suggest OsAMT1;1 may be essential for plant survival. This is curious results given the number of AMT1 genes present in rice and other species like Arabidopsis. It is assumed that most, if not all AMT1 genes in plants have overlapping physiological roles. The results presented suggest that not all AMT proteins are redundant or dispensable. It is also possible that during callus induction and regeneration, expression of antisense RNA decreased the endogenous target message and thereby prevented the synthesis of ammonium transporters. The small number of antisense transgenic lines are probably the ones having incomplete repression of endogenous ammonium transporters. This could have resulted from transgene position effect such as in or near repetitive DNA or heterochromatin (Prols and Meyer, 1992). If this were not to be the case, more lines with effective antisense co-suppression need to be generated, preferably using recently reported inverted repeat technology (Wang and Waterhouse, 2000).

The introduction of the OsAMT1;1 cDNA in sense orientation into the rice cultivars Taipei 309 and Jarrah under the control of the maize Ubi-1 promoter led to an increased accumulation of the mRNA. Transgenic plants with multiple copies of the transgene showed higher transcript levels. The positive correlation between OsAMT1;1 mRNA levels and transgene copy number suggested an additive effect of transgene copies, as observed previously using a reporter gene (Hobbs et al., 1993). Multiple T-DNA integrations are a common event with Agrobacterium-mediated transformation and there is conflicting evidence as to how such an increased copy number affects the expression of the introduced genes. The correlation between copy number and gene expression in transformants has been reported to be
positive (Gendloff et al., 1990), indeterminate (Shirsat et al., 1989) or negative (Hobbs et al., 1990). Differences in expression levels in lines with a single transgene copy may be due to transgene positional effects. For example a single transgene when inserted into the transcriptionally active region of euchromatin will produce higher expression due to the influence of the regulatory sequences of nearby host genes (Herman et al., 1990; Ker bundit et al., 1991; Koncz et al., 1989). On the other hand, if the transgene inserts in or near repetitive DNA or heterochromatin, transgene expression can be inactivated (Prols and Meyer, 1992).

6.3.1 OsAMTI;1 Transgenic Plants Showed Higher NH$_4^+$-Dependent Membrane Depolarisation

The membrane electric potential ($E_m$) of plant root cells is the driving force for ammonium uptake (Smith and Waker, 1978; Ullrich et al., 1984; Wang et al., 1994). Ammonium uptake by plant cells generally leads to depolarisation of the plasma membrane, the magnitude of which depends on the rate of NH$_4^+$ uptake. Rice seedlings (21-27 d old) acclimatised in nitrogen free solution for 1 or 2 d exhibited a rapid depolarisation of root cell membrane electrical potential upon addition of ammonium. Transgenic lines (e.g. line 46) that over-expressed OsAMTI;1 showed mean depolarisation values nearly twice that of control plants. This presumably reflected the involvement of more transporters in the transgenic plants. Estimated half-saturation values for net depolarisation ($K_m$) were similar in N acclimatised wild type and transgenic plants despite the differences in maximum depolarisation values ($V_{max}$). This indicated that ammonium uptake in the wild type is achieved by OsAMTI;1 or another AMTI family member with a similar affinity for ammonium.

6.3.2 Does High NH$_4^+$ Accumulation in OsAMTI;1 Overexpressing Lines Lead to Ammonium Toxicity in Transgenic Plants?

In this study, under adequate N nutrition, OsAMTI;1 overexpressing plants had (i) higher NH$_4^+$ uptake rates; (ii) higher NH$_4^+$ concentration in the roots; and (iii) lower biomass in comparison to control plants. However, biomass and root ammonium contents remained the same in both transgenic and wild type plants when plants were grown for 4 weeks at a very low concentration of ammonium (10 $\mu$M). The higher NH$_4^+$ uptake rate, increase in root NH$_4^+$ pool and reduction in biomass, in over-expressing plants positively-correlated with transgene copy number and OsAMTI;1 mRNA expression levels. Segregants (T$_2$) from multiple copy transgenic lines (Line 46 & line 38) were highly variable in terms of plant phenotype. Some of the dwarf type plants from line 46 did not survive. It is conceivable that the excess accumulation of free NH$_4^+$ in overexpressing plants was toxic. Presumably ammonium uptake
by these plants exceeded their capacity to assimilate the ammonium. Interestingly, NH$_4^+$ efflux from root cells appeared to be insufficient to remove the excess ammonium. The biochemical bases for ammonium toxicity or tolerance in plant species is not yet fully understood. High ammonium can affect both morphology and physiology of plants. At the biochemical level NH$_4^+$ toxicity blocks ATP production and reduces CO$_2$ fixation in the chloroplast (Ikeda and Yamada, 1981; Puritch and Baker, 1967), and reduces starch synthesis (Marwaham and Juliano, 1976). High ammonium uptake may also prevent water movement from root to shoot, and as a result plants may wilt and die (Anderson et al., 1991) as was the case for some T2 segregants from line 46. Plants may avoid the toxic effects of ammonium by storing excess ammonium in ‘safe’ intracellular locations and by rapid consumption of ammonium in the cytoplasm or plastids. Data obtained by different techniques show that NH$_4^+$ concentrations may range between 6-18 mM in ear components of wheat (Maheswari et al., 1988), 3-8 and 10-20 mM, respectively in cytoplasm and vacuoles of maize root cells (Lee and Ratcliffe, 1991), up to 40 mM in the cytoplasm of rice and spruce roots (Kronzucker et al., 1995; Wang et al., 1993), up to 2 mM in xylem of barley, wheat and maize plants (Cramer and Lewis, 1993; Mattsson and Schjoerring, 1996) and up to about 2 mM in the apoplast of B. napus leaves (Husted and Schjoerring, 1995). The NH$_4^+$ concentration in plant tissues can thus in many cases be quite substantial. The mechanism by which plants avoid the accumulation of exceedingly high NH$_4^+$ concentrations is still not yet fully understood.

The enzymes involved in the assimilation of NH$_4^+$ into the nitrogen transporting amino acids (glutamine, glutamate, aspartate, and asparagine) are cytosolic and chloroplastic glutamine synthetase (GS1 and GS2), NADH, ferredoxin dependent glutamate synthase (NADH-GOGAT and Fd-GOGAT), aspartate aminotransferase (AspAT), and asparagine synthetase (AS) (Lam et al., 1995). Effects of variations in external N supply on expression of GS isoforms have been investigated in several studies with contradictory results. Kozaki et al., (1992) concluded that NH$_4^+$ activated the GS2 promoter of rice. GS2 polypeptide and mRNA did not change in Phaseolus vulgaris following NH$_4^+$ addition (Cock et al., 1990). Induction of GS by NO$_3^-$ has been proposed (De la Haba et al., 1992). GS isoforms from barley leaves and roots were fully active not only in NH$_4^+$ and NO$_3^-$ grown seedlings but also in N free grown plants (Mack, 1995), thus indicating that external nitrogen was not necessary for the induction of the barley GS isoforms. The conflicting reports concerning effects of nitrogen supply on GS activity may be due to the fact that carbon and nitrogen metabolism are regulated by dynamic changes in C/N ratios and by changes in metabolic status (Lam et al., 1995). Decreases in C or
N resources upregulate genes involved in their acquisition while abundance of these resources induces genes associated with use and storage (Lam et al., 1995). Nitrogen assimilation in Arabidopsis is regulated by changes in metabolic status. Light and sugars, both of which increase C/N balance, up-regulate the expression of genes involved in ammonia assimilation into glutamine and glutamate by chloroplastic GS and Fd-GOGAT, while they repress asparagine synthetase (AS) expression (Lam et al., 1996). Developmental events also appear to be important for regulation of the different GS isoforms. In barley while GS1a was already active in germinating seed, GS1b and GS2 became active several days later in the young seedlings (Mack, 1995).

There are differences between different plant genera in the inherent capacity to assimilate exogenously supplied ammonium. It has been shown in the past that older mustard seedlings (Sinapis alba L.) can assimilate excessive supplied nitrogen (5-30 mM NH$_4^+$) by avoiding excessive levels of internal ammonium. In contrast, seedlings of Scots pine (Pinus sylvestris L.) accumulated NH$_4^+$ in cotyledons and roots and showed no stimulation of GS activity after the application of NH$_4^+$. In addition, root growth was drastically reduced in Scot pine (Vollbrecht et al., 1989). Ammonium is often the preferred source for conifers like Pinus sylvestris, but is normally only present in low concentrations in soil (Adams and Attiwill, 1982; Cole, 1981). Conifers that are well adapted to such conditions may have intrinsically low levels of ammonium assimilation and insufficient capacity to assimilate high concentrations of exogenously supplied ammonium. This could explain the deleterious effects of high ammonium on young seedlings. The two diverse cultivars used in this study also showed inherent differences in ammonium uptake and assimilation, as manifest by their differential response to N nutrition.

The results obtained from this study indicate that the manipulation of the source, ammonium transport, in rice plants is not sufficient to increase ammonium uptake and utilisation. Simultaneous manipulation of N assimilatory pathways including manipulation of the metabolism in the sink tissues themselves, such as shoots and seeds may facilitate improved NH$_4^+$ utilization and growth in plants. It has been shown that tobacco plants overexpressing pea cytosolic GS1 had considerable growth advantage over wild type plants. In contrast, plants co-suppressed for both chloroplastic and cytosolic GS had decreased GS activity (T. Brears and G. Coruzzi, unpublished results, cited by Lam et al., 1995) and caused plants to grow poorly. Therefore, this indicates that GS could be a rate limiting enzyme in plant
growth and nitrogen use. Transgenic tobacco plants overexpressing the asparagine synthetase (AS) gene showed increased levels of free asparagine in leaves (Brears et al., 1993). By increasing the sink for ammonium, via increases in GS and/or other N assimilating enzymes, it may be possible to avoid the deleterious effects of AMT1 overexpression in rice. In that case, it may well be possible to enhance N use efficiency and growth in rice in the future.

6.4 Future Work

From the above discussions it is clear that expression of different isoforms of rice GS under different nitrogen conditions need to be studied in OsAMT1;1 overexpressing lines to understand more about ammonium transporters and ammonium assimilation pathways. It will be very interesting to study the effect of overexpression OsAMT1;1 on GS and other downstream metabolic enzymes involved in ammonium assimilation and storage. This may be possibly by creating lines that overexpress both AMT and GS. It will also be interesting to know the effect of OsAMT1;1 overexpression on the carbon skeleton and the C/N ratio of the plants grown under different nitrogen conditions. Another important question that needs to be addressed is; "Does an increase in accumulation of NH₄⁺ in transgenic plants inhibit CO₂ fixation due to inhibition of photosynthesis?" (Platt and Anthon, 1981). There are some reports (Ikeda and Yamada, 1981; Walker et al, 1984; Johansson and Larsson, 1986) showing no correlation between high accumulation of NH₄⁺ and inhibition of photosynthesis. To understand the environmental and developmental regulation of ammonium transport activity and the tissue specific expression of this gene, a promoter–gus fusion analysis also needs to be carried out. Detailed analysis of lines with OsAMT1;1 promoter–gus transgenes will unravel environmental, spatial and developmental regulation of these genes.
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